flippases have been implicated in human diseases that include intrahepatic cholestasis, Angelman syndrome, autism, Tangier disease, macular dystrophy and adrenoleukodystrophy.

What is the outlook for researchers working on flippases? Not too bad. Cells contain a whole battery of flippases and these activities can be increasingly attributed to specific proteins. Establishing the primary function of candidate flippases and how they contribute to cell function and human disease is becoming a central issue in biology.

Where can I find out more?

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Human cilia proteome contains homolog of zebrafish polycystic kidney disease gene qilin

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Cilia are found on most cells of the body and are thought to play important roles in physiology and development. Not surprisingly, it is now understood that a very wide range of human diseases and developmental defects results from defects in ciliary assembly or function [1]. The steadily increasing number of disease genes related to cilia suggests that identification of ciliary proteins is a productive way to identify new candidate disease genes. Recent bioinformatics studies have followed this strategy by using comparative genomics to identify likely ciliary genes found only in organisms that have cilia and flagella. This ingenious approach has turned out to be highly productive, most notably by revealing the identity of the Bardet-Biedl Syndrome BBS5 gene [2]. In a recent *Current Biology* Dispatch [3], Greg Pazour presented a carefully thought-out analysis of these approaches, and after discussing several potential limitations, concluded that there is an urgent need for proteomic analysis to determine directly which proteins are contained in cilia and flagella.

In fact, a proteomic analysis of human cilia was completed and published two years ago [4], but curiously this prior work is not referred to, either in the Dispatch on the subject or in the recent comparative genomics studies of flagella. One likely reason for this is that the list of candidate proteins consisted mostly of hypothetical proteins or proteins with no obvious connection to ciliary structure or function, raising the specter, present in all proteomic analyses, of cross-contamination with undesired proteins. However, the human cilia proteome was annotated strictly by searching databases of human sequences, whereas the vast majority of the work on cilia and flagella composition has been done in other model organisms. Inspired by Pazour's article, we re-assessed the annotations for the proteins in the human cilia proteome, by comparing each published entry to the *Chlamydomonas* genome sequence. We chose *Chlamydomonas* as the reference organism because by far the most is known about flagellar components in this particular system.

By searching the *Chlamydomonas* genome using the sequences from the human cilia proteome, we found that 31 of the previously uncharacterized proteins can now be recognized as homologs of either *bona fide* ciliary proteins or proteins identified in comparative genomics analysis of flagellar genomes (Table 1). These include intraflagellar transport proteins, axonemal structural proteins, and proteins required for motility. The presence of a significant number of proteins known to be important for ciliary assembly and function increases our confidence in the validity of these published proteome data, and raises the possibility that some of the other uncharacterized genes could be candidate genes for cilia-related diseases.

A recent forward genetic screen in zebrafish has revealed three novel genes, mutants of which cause polycystic kidney disease (PKD) [5]. Although the PKD phenotype suggested a potential involvement of cilia, only one gene found in the screen showed an obvious ciliary defect. The question was thus posed whether the remaining two genes, *seahorse* and *qilin*, were somehow involved with cilial function. Importantly, upon detailed re-examination, we found that the human cilia proteome contains a homolog of

Table 1. Re-annotation of human cilia proteome entries based on current *Chlamydomonas* genome sequence information.

Protein ID	Prior annotation	*Chlamydomonas* homolog
27	Nucleoside diphosphokinase	**RSP23 (NDPK)**
32	Novel protein isoform 1	FABP 165606
50,121,199	cDNA FLJ10466	**Rib72**
51,211	KIAA1374 protein	**IFT80/Che-2**
69,133	Carnitine deficiency protein CDV1	**IFT81**
76	hsp89-α-Δ-N	**Hsp90A (flagellar)**
87	Glutathione S-transferase P	FABP 152547
105	Calcyphosine	FABP 164549
106	Nedd5/Septin KIAA0158	FABP 171634
126	Hypothetical protein	**ODA1**
127	Hypothetical protein	**IFT172**
128	Hypothetical protein	**Beta tubulin**
134	KIAA1023	FABP 167749 (asp)
136	CG9492 protein	**ODA2**
137	KIAA0944	**Dynein arm heavy chain**
139	Hypothetical protein	**Rib43a**
140	Hypothetical protein	FABP 162272
141	NM23-H7	**RSP23 (NDPK)**
163	Dpy-30 like protein	FABP 167776 (Dpy-30)
178	T27E7.6 protein	**PF2**
181	Sperm protein SPAG6	**PF16**
183	Growth arrest protein	**PF2**
196	Novel protein	FABP 162703 (PACRG)
200	cDNA FLJ14117	FABP 161253
202	KIAA1640	FABP 160383
206	Hypothetical protein	FABP 159749
207	Hypothetical protein	FABP 154065

The 'ID'-column gives identifier numbers listed in the combined results table (Table II) of [4], followed by the protein description given in that paper. '*Chlamydomonas* homolog' indicates results of BLAST searching of the *Chlamydomonas* genome v.2 (http://genome.jgi-psf.org/chlre2/chlre2.home.html). Proteins listed in bold are known flagellar components in *Chlamydomonas*. Remaining proteins are components of the *Chlamydomonas* 'Flagellar and Basal Body Proteome' identified by comparative genomics [2] and are listed according to the *Chlamydomonas* genome identification number.

qilin, which was referred to in the initial proteome paper as 'hypothetical protein KIAA0643' (BLAST comparison with *qilin* yields an *E* value of 10^{-134}). We therefore conclude that qilin represents a ciliary protein whose defects can cause polycystic kidney disease.

Another very interesting protein found in the cilia proteome is the human homolog of Dpy-30, a gene product involved in dosage-compensation in *Caenorhabditis elegans*. Although Dpy-30 is primarily known for its role in dosage compensation, *dpy-30* mutants also show defects in mating behavior, which is known to involve sensory cilia in the tail [6]. It would thus be extremely interesting to explore whether Dpy-30 plays any direct role in the sensory cilia of male worms.

This simple analysis further strengthens the connection between cilia and PKD, but most importantly reinforces the validity of genomic and proteomic methods to identify ciliary components and as a way to locate new disease genes. Moreover, the fact that many *bona fide* cilia/flagella proteins went unrecognized in the cilia proteome underscores the importance, as well as the challenges, of mining useful data from large scale proteomic experiments and integrating them with functional data from other systems. Because flagella are most thoroughly understood in *Chlamydomonas*, it is likely that the analysis of the flagellar proteome of *Chlamydomonas* currently underway [7], will serve as a 'Rosetta Stone' for deciphering other ciliary proteomes, and the community anxiously awaits the formal release of this data.

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